Development of the vertebrate small intestine and mechanisms of cell differentiation

MICHEL DAUÇA[1]*, FRANÇOISE BOUZIGES[2], SUZANNE COLIN[1], MICHÈLE KEDINGER[2], JEAN-MARIE KELLER[1], JOSEPH SCHILT[1], PATRICIA SIMON-ASSMANN[2] and KATY HAFFEN[2]

[1]*Laboratoire de Biologie Cellulaire du Développement, Université de Nancy I, Vandoeuvre-les-Nancy,* and [2]*INSERM, U 61, Biologie Cellulaire et Physiologie Digestive, Strasbourg, France*

ABSTRACT The intestinal epithelium represents an attractive biological model of differentiation from stem cells to highly differentiated epithelial cells, not only during particular developmental events depending upon the vertebrate species considered but also throughout adult life. The ontogenic maturation of the intestinal epithelium arises from both a programmed expression of specific genes and epigenetic influences mainly due to epithelial and mesenchymal interactions and hormonal participation. In the present paper we review the structural and functional changes that occur in the amphibian, avian and mammalian intestine during embryonic and/or post-embryonic development. Furthermore, we review the data concerning the mechanisms which control the cytodifferentiation of the intestinal epithelium.

KEY WORDS: *cell interactions, enterocyte differentiation, brush-border enzymes, cytoskeletal proteins, extracellular matrix*

Introduction

The embryonic origin of the intestinal tract is similar in all vertebrates. The endoderm gives rise to the epithelial sheet which lines the intestinal lumen, while the mesenchyme provides the connective tissue or *lamina propria* which supports the epithelium and forms with the latter the mucosa and the outer strata, i.e., - the submucosa - the muscularis made up of two muscular strata - and the serosa. It is worth noting that the intestinal epithelium is separated from the *lamina propria* by a continuous basement membrane and a complex extracellular matrix whose components are synthesized and secreted by both epithelial and underlying fibroblastic cells. Depending upon the vertebrate species considered, large variations are found in the relative importance of the outer parietal layers. Differences also exist in the morphological organization of the mucosa. Indeed, while longitudinal mucosal folds are characteristic of the intestines of all adult fish, amphibians and reptiles, they are replaced in birds and mammals by villi. The development of these various mucosal projections increasing the absorptive surface area of the epithelium will be examined in the present review including both lower (amphibians) and higher (birds and mammals) vertebrates.

The constituent cells of the intestinal epithelium display complex morphological specializations suitable for the large range of specific intestinal functions. As the absorptive cell is the main cellular type of the intestinal epithelium, we will analyze the main features of this highly differentiated cell. Shifts in feeding behavior often occur during the development of vertebrates and are reflected at the intestinal level by changes in the molecular components of the absorptive cells. In our laboratories, we have studied in detail the ontogenesis of the intestinal digestive hydrolases during amphibian, avian and mammalian development. Data obtained are discussed below.

The multistep process of intestine morphogenesis and intestinal cell differentiation proceeds in a specific temporal and spatial pattern. In the present paper we will emphasize that the ontogenic maturation of the intestinal epithelium arises from both a programmed expression of specific genes in endoderm and epigenetic influences mainly due to reciprocal epithelial-mesenchymal tissue interactions in which extracellular matrix molecules appear to play important roles, and from hormonal participation. In this review, special attention will be paid to results in recent years from our own research and that of others concerning the mechanisms controlling the cytodifferentiation of the intestinal epithelium.

Morphologic development of the intestine

During anuran amphibian development, microphagous or herbivorous feeding of the larva is superseded by carnivorous feeding

Abbreviations used in this paper: BM, basement membrane; ECM, extracellular matrix; EGF, epidermal growth factor; DX, dexamethasone.

***Address for reprints**: Laboratoire de Biologie Cellulaire du Développement, Université de Nancy I - Faculté des Sciences - BP 239, F-54506 Vandoeuvre-les-Nancy Cedex, France

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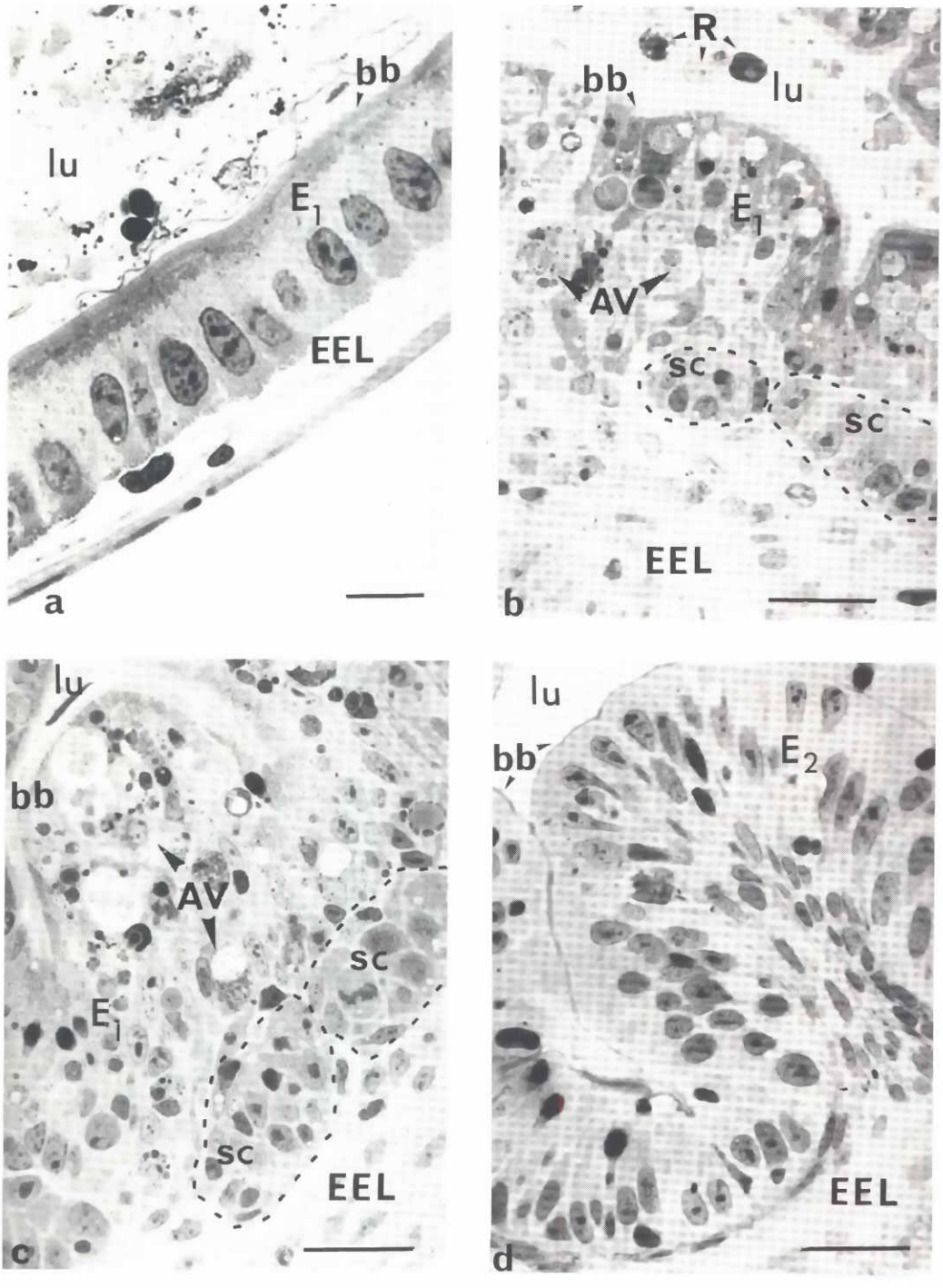

Fig. 1. Histological changes in amphibian intestine during spontaneous metamorphosis. *During the larval period* **(a)**, *the intestinal wall is mainly composed of the flat primary epithelium (E1) which exhibits a typical brush border (bb). The extraepithelial layers (EEL) are thin. During metamorphosis* (**b** and **c**) *(beginning and end of climax respectively) the primary epithelium degenerates. Autolytic vacuoles (AV) are clearly seen in the columnar larval absorptive cells which have become pyknotic. Cell remnants (R) are sloughed off into the intestinal lumen (Lu). Proliferation of stem cells (SC) gives rise to nests (—). At the end of metamorphosis* **(d)**, *cell nests have coalesced to form the secondary intestinal epithelium (E2) which presents some folds. Bars: 10 μm.*

in the post-metamorphic froglet and adult. These functional changes are paralleled by significant histomorphological alterations and remodeling of the intestinal tract. At climax of metamorphosis, the intestine shortens considerably (Dauça and Hourdry, 1981), loses its coiled shape and shows striking cellular degeneration and regrowth in different regions (Fig. 1). Following a characteristic pattern of epithelial lysosomal activity and change, the larval (primary) epithelium degenerates and the remains of the tissue are extruded into the lumen and ejected (Hourdry, 1969). The secondary epithelium with its complex system of longitudinal folds is formed by the proliferation of stem cells at the base of the primary epithelium (Mc Avoy and Dixon, 1977; Dauça and Hourdry, 1978). As the folds increase in number and in height, mitoses become confined to the troughs and a gradient of decreasing mitotic activity from troughs to crests is formed. It has been suggested that the nests represent a primitive form of intestinal crypt (Patten, 1960).

The turnover time for the anuran intestinal epithelium is approximately 16 days. Amphibian intestinal epithelial cells therefore have a considerably longer life span than those of higher vertebrates which complete the journey from the crypts to the villous tips in one to six days. Following proliferation of cell nests, differentiation of daughter cells leads to three main epithelial cell classes, i.e. absorbing, goblet and endocrine cells (Mc Avoy and Dixon, 1978a,b); no Paneth cells are found.

The small intestine of 5 to 6-day chick embryos is lined by an undifferentiated stratified columnar epithelium which cannot be distinguished from the epithelia of other regions of the digestive tract. After day 6, the inner surface of the midgut of chicken embryos shows longitudinal folds. By day 11, some folds take a zigzag appearance. Thereafter these previllous ridges running along the length of the intestine give rise to the villi progressively covered by a single layer of columnar epithelial cells (Grey, 1972; Lim and Low, 1977; Bryk *et al.*, 1979; Chambers and Grey, 1979).

The time of appearance of mucosal villi, crypts and folds and of the various epithelial cell types during morphogenesis of the mammalian small intestine have been investigated extensively and reported by many authors (Dunn, 1967; Deren, 1968; Koldovsky, 1969; Vollrath, 1969; Mathan *et al.*, 1976; Trier and Colony Moxey, 1979; Bailey *et al.*, 1984; Lacroix *et al.*, 1984a). The main morphogenetic events can be summarized as follows: at early fetal stages, the intestinal anlage consists of a single tube composed of radially arranged stratified epithelial cells – the endoderm (future absorptive epithelium) – surrounded by undifferentiated mesenchymal cells (future muscle layers and connective tissue). Thereafter primitive villi are formed and lined by one layer of cells. During the fetal period, cell proliferation occurs in epithelial cells along the entire length of the villi, while after birth the mitotic activity is confined to the lower regions of the crypts (Hermos *et al.*, 1971). The progeny of these cell divisions migrate into the upper crypts where differentiation begins and then continues as cells emerge in ordered array out of the crypts, onto and along the villi (Lipkin, 1981). While absorptive cells are predominant, other epithelial cell types such as Paneth (found only in the base of the crypts), goblet, enteroendocrine, caveolated and M cells are also present in the intestinal epithelium (Fig. 2, Haffen *et al.*, 1986). Once they reach the tip of the villi, the cells are extruded into the intestinal lumen. In adult mammals, cell proliferation is an essential process in the continuously renewing epithelium of the small intestinal mucosa. The pioneering studies of Cheng and Leblond (1974) in the mouse species led to the concept that crypt stem cells give rise to the differentiated cell types of the villus epithelium. Recent investigations gave experimental support to this unitarian theory. Indeed, experiments involving the construction of chimeric mice composed of cells of two different genotypes exhibiting strain-specific markers demonstrated that each intestinal crypt is composed of a single genotype derived from a single progenitor cell (Ponder *et al.*, 1985; Kusakabe *et al.*, 1988). Similar evidence was obtained by the use of a marker induced by somatic mutation (Winton *et al.*, 1988) or the transgenic mice model (for review see Gordon, 1989).

Differentiation of enterocytes

Absorptive cells called enterocytes make up about 90% of the epithelial population of the vertebrate intestine, therefore detailed descriptions of their structural and biochemical characteristics have been given. We will focus our study on the main features of the differentiation of this cell type.

Development of the brush border

The apical membrane of absorptive intestinal cells displays a striking structural organization. The intestinal brush border consists of a densely-packed array of microvilli (Maroux *et al.*, 1988). This cell surface specialization is obviously well adapted for exchanges as the existence of ordered microvilli increases the cell surface area. The shape of the brush border is ensured by a particularly well-developed and organized cytoskeleton (Mooseker, 1985). Each microvillus contains an axial bundle of actin microfilaments anchored at the tip of the microvilli and bridge laterally by short thin filaments disposed regularly along the longitudinal axis of the microvillus (Mooseker and Tilney, 1975). Five major proteins have been found in the intestinal microvillar microfilaments: a 110-KDa protein, villin, fimbrin, actin and calmodulin. The core bundles of microfilaments contain actin (Tilney and Mooseker, 1971) and two actin-binding proteins, villin and fimbrin (Bretscher and Weber, 1979, 1980). The lateral bridges are made of the 110-kDa protein and calmodulin (Matsudaira and Burgess, 1979; Howe *et al.*, 1980).

Ultrastructural studies on the development of the intestinal epithelium of amphibians (Bonneville and Weinstock, 1970), birds (Overton and Shoup, 1964; Chambers and Grey, 1979) and mammals (Overton, 1965; Merril *et al.*, 1967) have revealed that the assembly of the brush border cytoskeleton is a complex and gradual process which needs spatial and temporal relationships between cytoplasmic vesicles, filamentous meshworks and apical membrane. More recently, the expression of microvillar core proteins has been examined in the developing vertebrate intestine by immunochemical and light microscopic immunolocalization techniques. It has been shown that villin is expressed before the assembly of the brush border takes place.

At climax of amphibian metamorphosis, the protein is detected in the undifferentiated basal stem cells of the developing secondary intestinal epithelium (Fig.3, Figiel *et al.*, 1987).

Villin displays concentrated apical staining in embryonic intestinal mucosa of the day 8 chick. At this time there are few microvilli on the intestinal surface and both fimbrin and the 110-kDa protein are distributed diffusely throughout the cytoplasm of the cell and segregate to brush borders only late in development, i.e the day before hatching (Shibayama *et al.*, 1987).

In rodents, significant villin gene expression can be demonstrated early during embryogenesis. Villin is first detectable at the early post-implantation stage in visceral endodermal cells at the periphery of the egg cylinder. In this extra-embryonic layer, the expression of villin increases and then persists until full term gestation. In the embryo, villin first appears in immature cells of the gut anlage as soon as the anlage appears (Maunory *et al.*, 1988). In addition, immature cells of the intestinal crypts of adult rats contain villin as do undifferentiated HT29 cells derived from a human colonic adenocarcinoma (Robine *et al.*, 1985). These data emphasize that villin is a precocious molecular marker for differentiating enterocytes. Similarly, two calmodulin-binding proteins, caldesmon and fodrin, are expressed in the cytoplasm of undifferentiated cells in the developing rat and human intestine. They segregate into the brush borders at the time of their differentiation (19-day fetal rat and 12-week human fetus (Rochette-Egly and Haffen, 1987; Rochette-

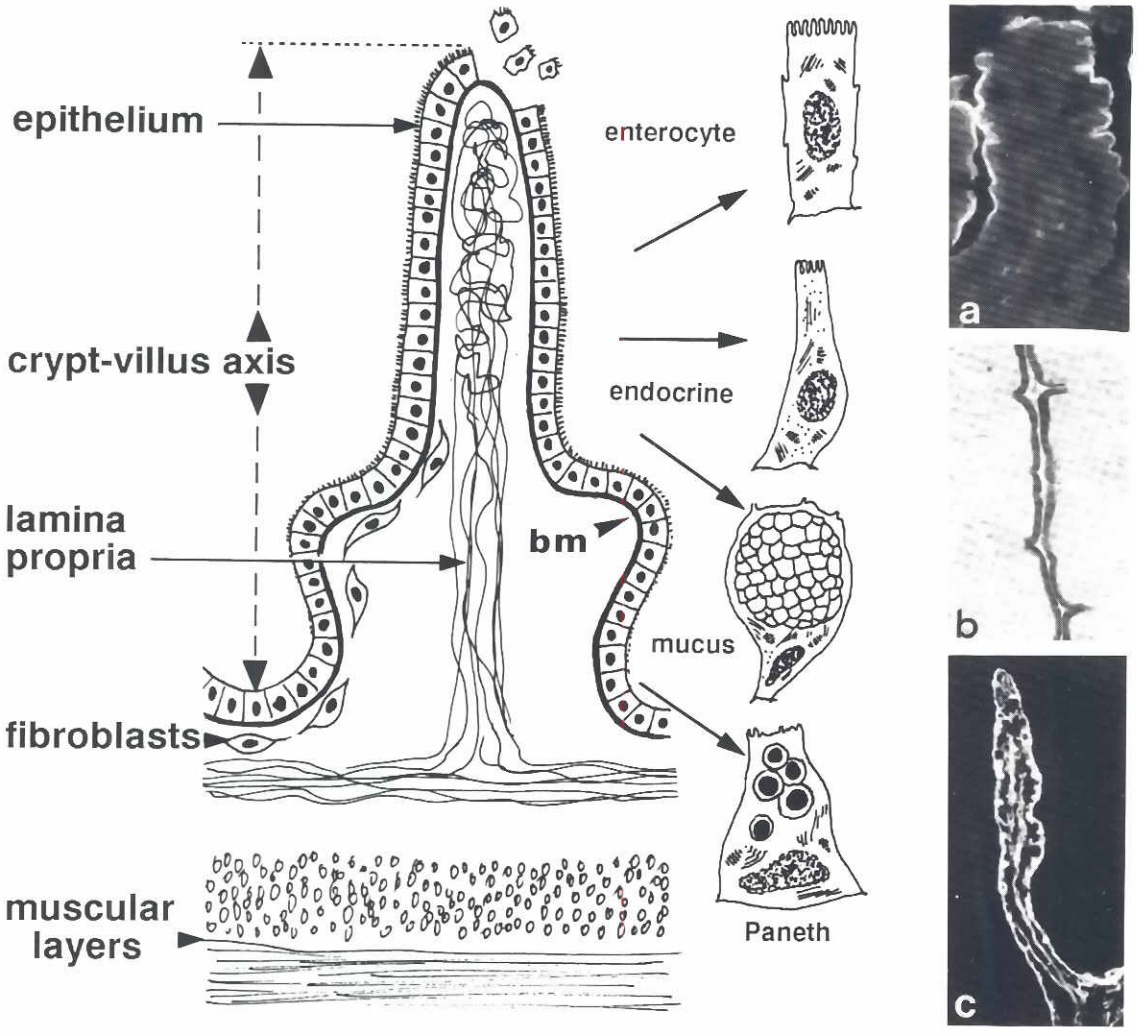

Fig. 2. Schematic representation of a cross section through the intestinal wall showing the various intestinal cell layers and epithelial cell types. *The immunocytochemical micrographs* **(a-c)** *emphasize on the one hand two markers of differentiated villus cells: a brush border membrane digestive hydrolase, aminopeptidase* **(a)** *and a protein of the brush border cytoskeletal core, villin* **(b)**, *and on the other hand basement membrane (bm) molecules underlying the epithelial cell layer such as laminin, all along the crypt/villus axis* **(c)**.

Egly *et al.*, 1988b). Concerning the developmental pattern of the 110-kDA protein, its first expression coincides with the time of definite brush border assembly but is preceded by the transient expression of a 135-kDA form in immature epithelial cells. A similar pattern of expression of these two latter proteins has also been observed during the differentiation pathway of HT-29 cells (Rochette-Egly *et al.*, 1988a).

Ontogenesis of intestinal digestive enzymes

A number of hydrolytic enzymes known to be associated with the brush border increase in activity as differentiation of enterocytes proceeds. They can be considered as late markers of the functional differentiation of enterocytes during development.

Glucosidases (maltase, trehalase, glucomylase), alkaline phosphatase and γ-glutamyltransferase are present in the microvillus membrane of the larval (Dauça *et al.*, 1979) and adult (Dauça *et al.*, 1980a) amphibian intestinal epithelium. The former and latter are devoid of ß-galactosidase and sucrase activities (Hourdry *et al.*, 1979). The replacement of the intestinal epithelium during spontaneous metamorphosis is accompanied by an increase in the specific activities of both hydrolases detected (Hourdry *et al.*, 1979). Comparison of the protein, glycoprotein and enzyme patterns after gel electrophoretic separation of SDS-solubilized microvillus membranes has revealed an increase in the number of protein bands during metamorphosis of the bullfrog (Dauça *et al.*, 1981). In addition, it appears that maturation process occurs for alkaline phosphatase during the post-embryonic development of amphibians since the electrophoretic mobility of the larval intestinal enzyme is always faster than that of the juvenile and adult intestinal alkaline phosphatase. Such modifications have also been reported in developing mammals (Miki *et al.*, 1977; Mulivor *et al.*, 1978; Uezato *et al.*, 1981; Tojyo, 1984).

In the chick intestine, the developmental profile of enzyme activities is characterized by a marked increase in maltase and alkaline phosphatase activities shortly before hatching. Contrary to what is observed in amphibians, sucrase and traces of lactase activities are detected in the intestinal brush border of the chick. The developmental pattern of sucrase is somewhat different from maltase and alkaline phosphatase. Indeed, after an increase similar to that of maltase between Days 15 and 17 of incubation, sucrase remains nearly constant until 1 day after hatching. From these data it is reasonable to admit that in the chick embryo,

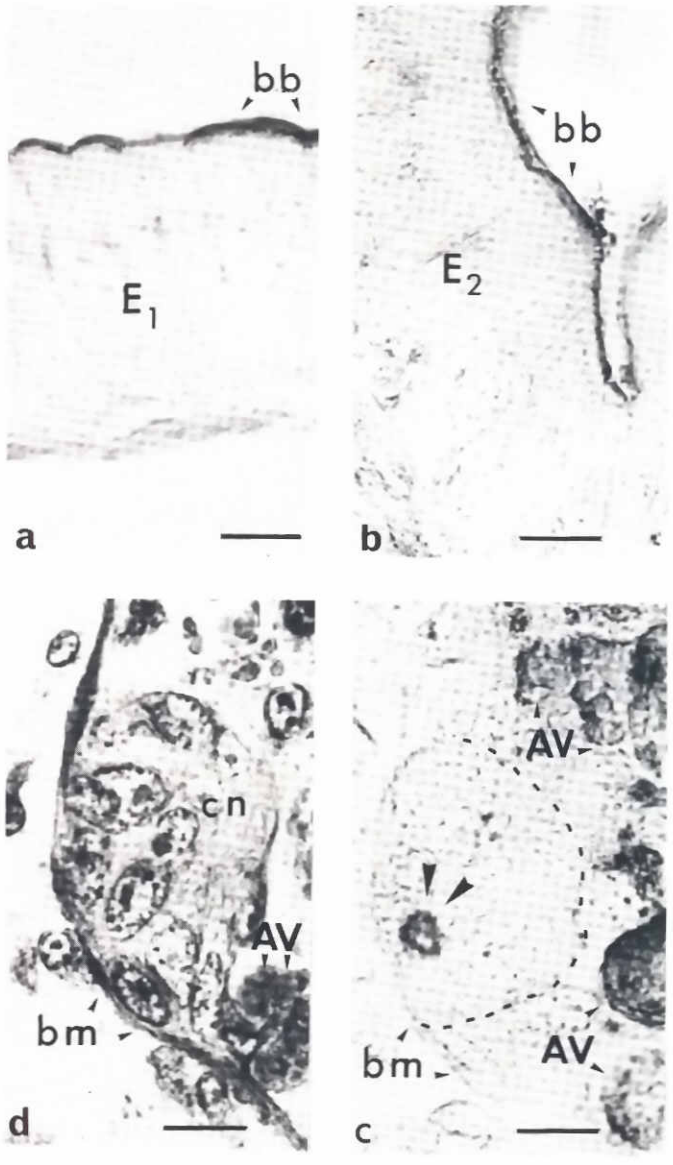

Fig. 3. Immunolocalization of villin in the metamorphosing amphibian intestine. *In the tadpole* **(a)** *as well as in the newly-metamorphosed juvenile* **(b)**, *the brush border (bb) of the flat primary (E1) and of the folded secondary (E2) intestinal epithelium is labeled. During metamorphosis the section of the climactic intestine stained with hematein eosin* **(c)** *exhibits cell nests (CN). The cytoplasm of an undifferentiated cell in the nest (—) is specifically labeled (arrow head). Dark areas covering the cell nest are due to the presence of autolytic vacuoles (AV) in the degenerating primary intestinal epithelium. bm: basement membrane. Bars: 10 μm.*

enzymatic differentiation of the small intestine is accomplished almost entirely at hatching (Moog, 1950; Siddons, 1969; Dautlick and Strittmater, 1970; Kedinger *et al.*, 1981) as it is in the amphibian intestine at the end of metamorphosis when the secondary epithelium has completely replaced the larval tissue.

In contrast, in rodents with short gestation periods like rats and mice, biochemically the intestine is relatively immature at birth and for the first two postnatal weeks. The definitive digestive and absorptive properties of the small intestine are acquired only during the third postnatal week (for reviews, see Moog, 1979a, b; Henning, 1979, 1981). This is demonstrated very nicely by the developmental pattern of lactase and sucrase. The rat lactase is detectable on day 18 of gestation, has its maximal activity at birth and then declines reaching adult values by the end of the fourth week (Doell and Kretchmer, 1962; Simon-Assmann *et al.*, 1982). During late gestation and early postnatal development, the rise of lactase activity parallels the increase in the amount of the corresponding mRNA (Sebastio *et al.*, 1989). In contrast, the decline of lactase activity observed at weaning occurs irrespectively to the constant rate of accumulation of the mRNA (Freund *et al.*, 1989) and has been correlated to modifications in the processing of the protein (Nsi Emvo *et al.*, 1987). On the other hand, sucrase cannot be detected in the intestine during the first and second postnatal weeks; its activity appears only around weaning, rises rapidly reaching adult levels by the end of the fourth week (Doell and Kretchmer, 1964; Rubino *et al.*, 1964; Reddy and Wostmann, 1966). During postnatal development, sucrase activity follows directly the amount of the corresponding mRNA accumulating in the enterocytes (Sebastio *et al.*, 1986).

The main changes in functional maturation of the small intestine in humans, characterized by a long gestation period and by a high degree of differentiation at birth, can be summarized as follows: a) the first phase of maturation, which occurs precociously during fetal life, mainly concerns the progressive proximo-distal onset and maturation of brush border enzymes like alkaline phosphatase, disaccharidases and peptidases and occurs from 8 weeks of gestation; b) a second important phase begins at 14 weeks, the stage at which the intestinal mucosa begins to be in contact with amniotic fluid. This second phase is marked by an important increase in almost all enzymes, aminopeptidase and sucrase reaching adult values; c) the third period takes place around the 6th month when lactase, maltase-glucoamylase and enterokinase, which had been very low or even absent, exhibit a pronounced upsurge (Grand *et al.*, 1976; Antonowicz and Lebenthal, 1977; Antonowicz *et al.*, 1977; Auricchio *et al.*, 1981; Lacroix *et al.*, 1984a). Messenger RNAs for lactase and sucrase present the same developmental profile in human as in the other mammals studied so far (Sebastio *et al.*, 1987, 1989).

Regulation of intestinal development

Research on the control mechanisms regulating the functional differentiation of the small intestine has emphasized the role that two kinds of environmental factors play: hormones and tissue interactions.

Hormonal control of brush border enzymes

Numerous investigations have been undertaken in order to clarify the precise role of hormones in the onset of digestive enzymes during ontogenesis of the vertebrate intestine. Comparative studies carried out on hormonal requirements indicate that considerable diversity exists among species in lower and higher vertebrates.

During anuran metamorphosis, the intestinal epithelium substitution is triggered by thyroid hormones. Indeed, epithelium re-

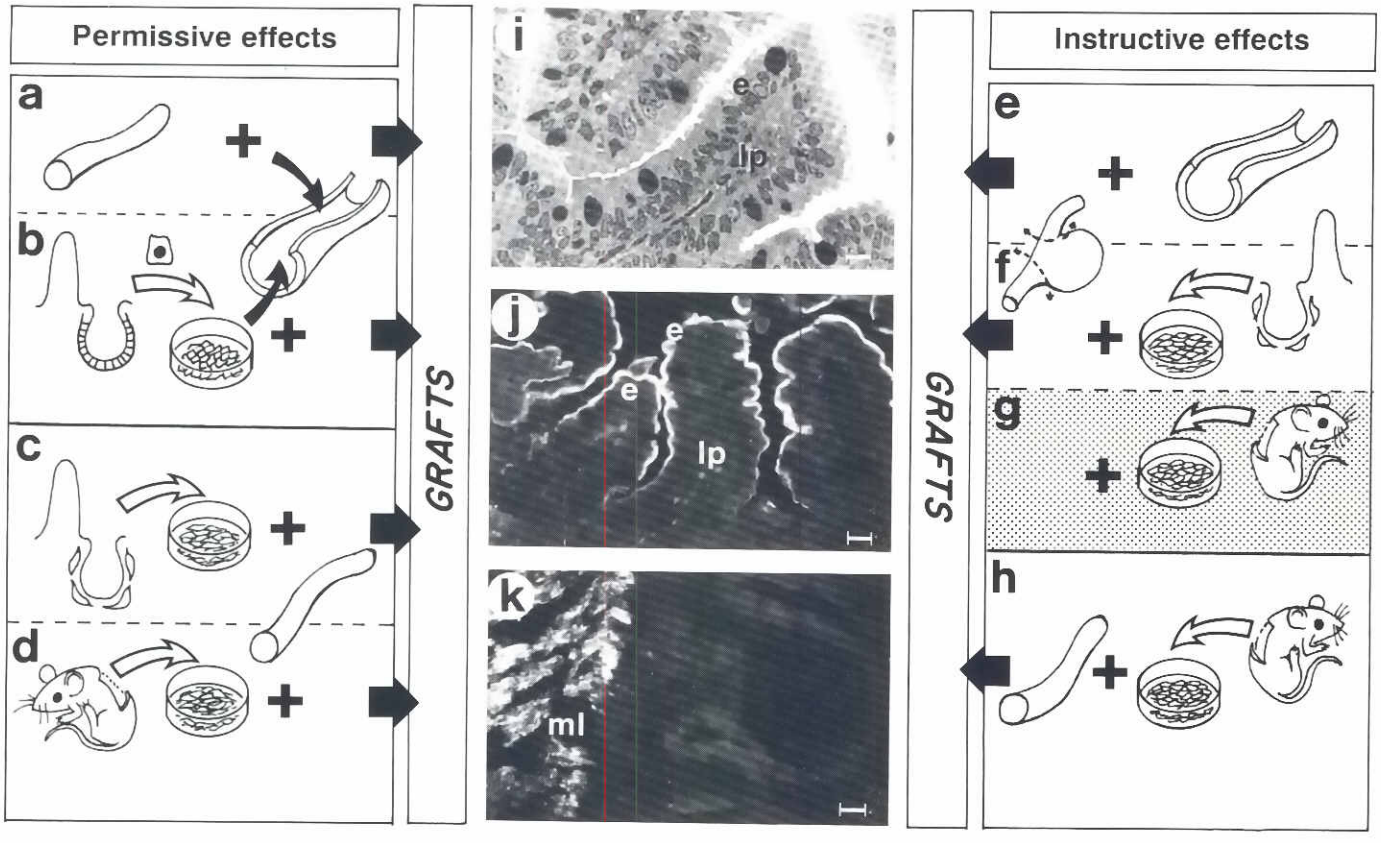

Fig. 4. Illustration of the various recombination experiments designed to demonstrate the existence of permissive (a-d) and instructive (e,f,h) interactions. *Permissive effects are displayed by fetal intestinal mesenchyme on the differentiation of intestinal endoderm* **(a)** *or adult crypt cells* **(b)** *as well as by postnatal intramucosal* **(c)** *or skin fibroblasts* **(d)** *on intestinal endoderm. Instructive effects are displayed by fetal intestinal mesenchyme* **(e)** *and postnatal intramucosal fibroblasts* **(f)** *on gastric endoderm which becomes intestinalized. This switch is not obtained with skin fibroblasts* **(g)**. *An other kind of instructive effect is displayed by intestinal endoderm, which induces the differentiation of skin fibroblasts into smooth muscle layers* **(h)**. *Micrographs* **(i-k)** *are representative of an intestinal morphogenesis and differentiation of the associations after grafting; light micrograph of a section stained with periodic acid Schiff* **(i)**; *immunodetection of brush border digestive enzymes at the apex of the epithelium* **(j)** *and of smooth muscle actin in the muscle layers* **(k)**. *e: epithelium; lp: lamina propria; ml: muscle layers. Bars: 20 μm.*

placement can be induced in thyrostatic larvae treated with exogenous thyroxine (Dauça and Hourdry, 1977) or triiodothyronine (Figiel *et al.*, 1989). However if thyroid hormones exert a direct proliferative effect on basal stem cells of the intestinal epithelium (Pouyet and Hourdry, 1980), they cannot lead alone to the differentiation of the brush border in the developing secondary tissue, as seen in intestinal explants taken from premetamorphic larvae and cultured *in vitro* for 10 days with thyroxine (Pouyet and Hourdry, 1977). In addition, after spontaneous metamorphosis and contrary to what is obtained after thyroid hormone-induced metamorphosis, the activities of glucidases increase in the newly-formed secondary intestinal epithelium (Hourdry *et al.*, 1979; Dauça *et al.*, 1980a, b). This difference has been interpreted as the consequence of more extensive endocrine changes during spontaneous metamorphosis than during hormonal treatment, including the inter-renal cells of the adrenal glands and the pancreatic islets of Langerhans (Hourdry and Beaumont, 1985). *In vivo* (El Maraghi-Ater *et al.*, 1986; Ben Brahim *et al.*, 1987) and *in vitro* (Pouyet and Hourdry, 1988) studies have led to the conclusion that thyroid hormones have no effect on hydrolase activities and that the maturation of the amphibian intestinal brush border is mainly under the control of glucocorticoids and to a lesser extent of insulin.

In birds, thyroid hormones and glucocorticoids are primarly responsible for the various intestinal changes that occur during late embryonic life. In organ culture of chick embryonic duodenum, addition of thyroxine accelerates morphologic and enzymatic differentiation of epithelial cells. It stimulates notably the growth of microvilli. It has a strong activating effect on alkaline phophatase and a milder effect on maltase activity, both of which are bound to the microvillus membrane (Black and Moog, 1978; Mallon and Betz, 1982). Thyroxine also induces glycosylation but not *de novo* synthesis of these two enzymes (Yoneyama and Moog, 1982). In addition, glucocorticoids favor the appearance of the brush border *in vitro* and increase the activities of associated enzymes, mainly sucrase and maltase (Moog and Richardson, 1955; Yoshizawa *et al.*, 1976, 1977; Black and Moog 1978). An action of the glucocorticoids at the gene level has been suggested by the fact that the addition of an inhibitor of messenger RNA transcription (actinomy-

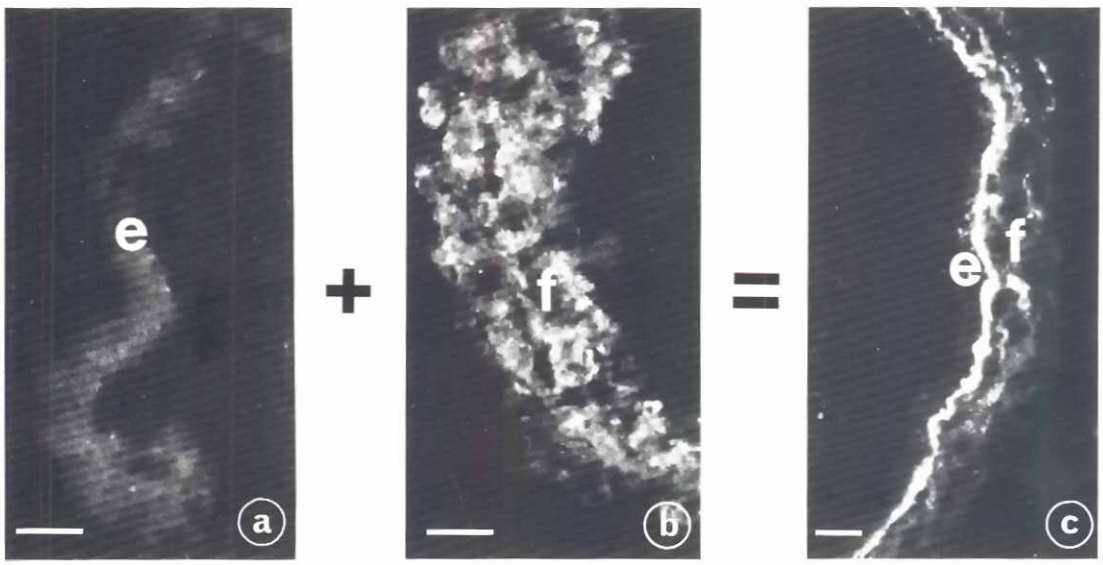

Fig. 5. Representative immunostaining of type IV collagen *in 5 μm cryosections of 14-day fetal intestinal endodermal* **(a)** *or mesenchymal* **(b)** *cell monocultures and of an epithelial-fibroblastic coculture* **(c)**. *No labeling is obvious in the endodermal cells in contrast to the fibroblastic cells where the BM molecules are found intra- and extracellularly. A polar deposition of type IV collagen at the epithelial-fibroblastic interface occurs only in the coculture conditions. e: endodermal cells; f: fibroblastic cells. Bars: 20 μm.*

cin D) to the culture medium prevents some of these activities from increasing (Yoshizawa *et al.*, 1977). However, recent studies have shown that in the intestine the hormonal response is mediated by epithelial-mesenchymal interactions. This aspect will be discussed later in the chapter.

There is now a great deal of evidence suggesting that the regulation of the morphological and functional maturation of the mammalian small intestine is under multihormonal control.

In vivo studies involving either hormonal treatments, endocrine organ ablations or dietary changes have shown that glucocorticoids are mainly required for the normal enzymatic maturation of the postnatal rodent intestine and have been the subject of extensive reviews (Moog, 1979a, b; Kedinger *et al.*, 1986a, b; Henning, 1987). If drastic changes in the enzyme activities of the rodent intestine are precociously induced by administration of glucocorticoids and delayed by adrenalectomy, enzyme molecular conversion may also be obtained during *in vivo* glucocorticoid treatment, as has been demonstrated for alkaline phosphatase (Tojyo *et al.*, 1985). In addition, the participation of thyroid hormones, insulin and epidermal growth factor (EGF) during postnatal maturation processes has also been suggested (Henning, 1978; Menard *et al.*, 1981; Malo and Menard, 1982, 1983).

Because organ culture removes the studied tissue from the interacting complexities of the intact organism, it permits a systematic approach to the search for the intrinsic and extrinsic regulators of intestinal epithelial maturation that is possible *in vivo*. In the following analysis we would like to integrate our own *in vitro* data on the respective specific and direct effects of different hormones on the brush border enzyme maturation of the rodent and human intestine.

- While pentagastrin (Lichtenberger *et al.*, 1973; Johnson, 1976), glucagon (Gleeson *et al.*, 1971) and EGF (Feldman *et al.*, 1978; Scheving *et al.*, 1980; Al Nafussi and Wright, 1982; Malo and Menard, 1982, 1983) have been reported to influence intestinal cell proliferation, on the other hand these mitogenic factors do not exert any effect on brush border enzyme expression of the rodent intestine (Simon *et al.*, 1982).

- If the onset of lactase in the fetal rodent intestine is initially mediated by an intrinsic potentiality of the endoderm, its transitory increase at birth is mainly under the control of glucocorticoids. Indeed, dexamethasone (DX) is able to increase prenatally lactase activity *in vitro* leading to values present *in utero* just before birth (Simon-Assmann *et al.*, 1982). The use in organ culture of transcription (actinomycin D) or of translation (cycloheximide) inhibitors have led to the conclusion that 50% of the increase in lactase activity before birth *in situ* is due to the expression of pre-existing mRNAs and that 50% is due to the action of glucocorticoids on the synthesis of new gene products (Simon-Assmann *et al.*, 1984a). The presence of triiodothyronine during 48 hours of culture provokes a significant inhibition of DX-stimulated lactase activity from 19 days of gestation onwards (Simon-Assmann *et al.*, 1984b). It is very likely that thyroid hormones are involved in the normal decrease in lactase activity at weaning, since during this period increased-concentration of circulating thyroxine is detected (Henning, 1981), and that thyroxine injections into 9-day-old rats induces a 50% decrease in the enzyme activity (Kedinger *et al.*, 1986b).

Sucrase and maltase activities are also prenatally sensitive to glucocorticoids (Simon-Assmann *et al.*, 1982; Beaulieu and Calvert, 1984, 1985). The precocious induction of sucrase could be elicited from day 19 onward whereas the stimulation of maltase could be obtained as early as day 18 (Simon-Assmann *et al.*, 1982). The presence of actinomycin D in the culture medium supplemented with DX is followed by different levels of inhibition related to the enzyme considered: 100% for sucrase and 70% for maltase (Simon-Assmann *et al.*, 1984a). These data suggest that the mechanism by which DX induces sucrase and stimulates maltase activity takes place at the transcriptional level. In previous studies it has been demonstrated that glucocorticoids enhance the glycosylation of pre-existing proteins (Kedinger *et al.*, 1980). These data also indicate that the basic maturation of maltase depends upon the translation of a pre-existing pool of mRNAs. It is worth pointing out that the glucocorticoid responsiveness of the fetal intestine is higher than during the postnatal period of life (Kedinger *et al.*, 1980). This phenomenon may be due to differences in receptor activities. Indeed, small bowel receptor activity is present before birth (Henning *et al.*, 1975), reaches a peak 1 day before birth and declines thereafter

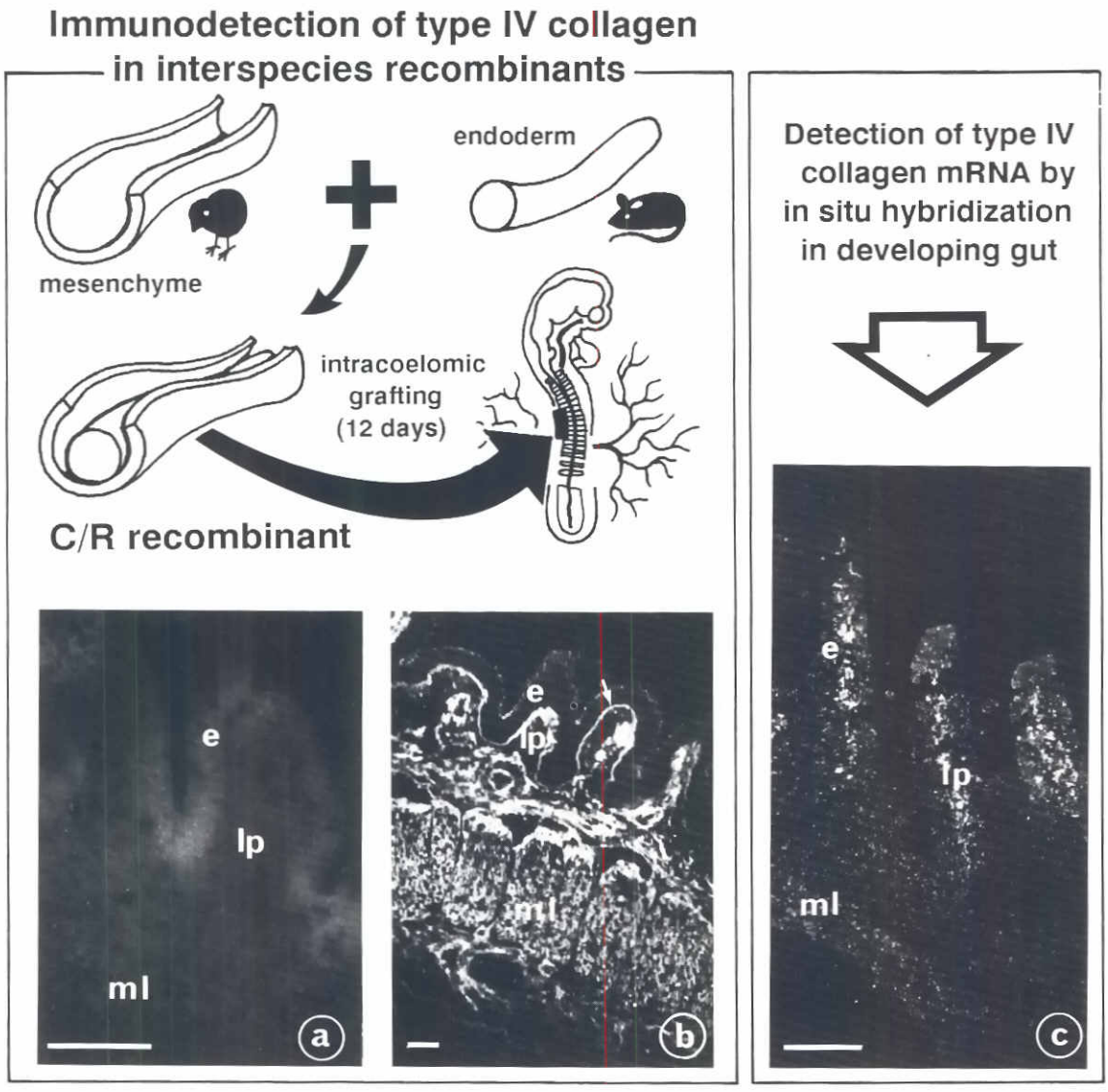

Fig. 6. Immunodetection of type IV collagen in a chick mesenchyme/rat endoderm recombinant after *in ovo* grafting, *with anti-rodent* **(a)** *or anti-chick* **(b)** *type IV collagen antibodies. Note that the epithelial/lamina propria interface (arrow) is labeled only with the latter antibody indicating the mesenchymal origin of this BM constituent. This result is confirmed by the accumulation of type IV collagen mRNA in the lamina propria of a 2 day-mouse intestine* **(c)**. *e: epithelium; lp: lamina propria; ml: muscle layers. Bars: 20 μm.*

(Lentze *et al.*, 1985). Concerning adrenal and plasma corticosterone concentrations, it has been reported that this hormone presents maximum levels at 18-19 days of gestation (Holt and Oliver, 1968; Kamoun, 1970; Cohen, 1973; Martin *et al.*, 1977). Thus, corticosterone of fetal origin is present at a high concentration at the time at which DX is able to produce the upsurge of intestinal enzyme activity *in vitro*. Other factors such as thyroid hormones, insulin, EGF and pentagastrin, known to exert *in vivo* a stimulating effect upon the enzymatic maturation of the fetal intestine, have been tested *in vitro* in organ culture. They were found to be devoid of any effect *per se* on sucrase and maltase activities (Simon *et al.*, 1982). Nevertheless the presence of a thyroid hormone in the culture medium led to a further stimulation of DX-induced sucrase (Simon-Assmann *et al.*, 1984b) and maltase (Beaulieu and Calvert, 1985) activities.

Differences have been noted among rodent species concerning the effect of DX on the level of alkaline phosphatase in the fetal small intestine in culture (Simon-Assmann *et al.*, 1982; Beaulieu and Calvert, 1985). Of particular interest is the fact that alkaline phosphatase activity is superinduced *in vitro* by actinomycin D, which supports the notion that an actinomycin D-sensitive repressor may play a role in the maturation process of this enzyme (Simon-Assmann *et al.*, 1984a). Such a mechanism has also been suggested by Moog and Wiemerslage (1981) to explain the development pattern of intestinal alkaline phosphatase in the chicken embryo before hatching.

It is clearly of importance to explore the regulatory factors involved in the enzymatic maturation of the human fetal intestine. The difficulty in obtaining fetuses before 8 weeks of gestation made it impossible to study the factors that might influence the onset of the brush border enzymes present in the human fetal gut as early as this stage. One can nevertheless speculate that, as in rodents (Kedinger *et al.*, 1981), the first expression of enzymes in the human fetal intestine is an intrinsic property dependent upon tissue interactions. Indirect evidence of this assumption is provided by the fact that human fetal gut mesenchyme exerted an inductive action

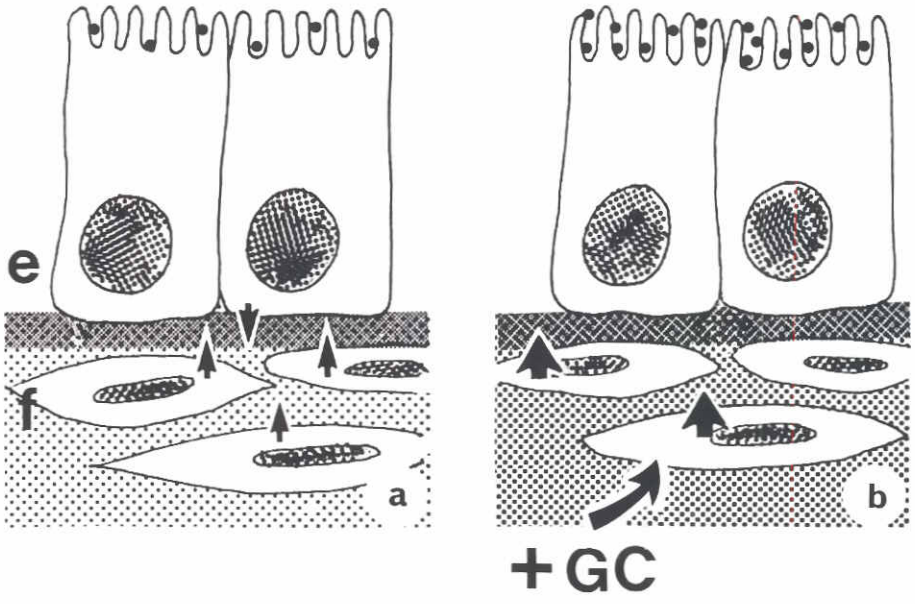

Fig. 7. Schematic representation of the proposed mechanisms of epithelial-fibroblastic cell interactions (a) and of glucocorticoid (GC) action (b) for intestinal cell differentiation processes. *In* **(a)** *the heterotypic cell contacts induce modifications in the ECM microenvironment and in particular the elaboration of a basement membrane, whose constituents are produced by both cell populations as a function of the cell type. In* **(b)**, *glucocorticoids act on the fibroblasts, eliciting modifications in the synthetic activity of ECM molecules leading to a new microenvironment that in turn could induce an accelerated epithelial maturation. e: epithelial; f: fibroblastic cells.*

on sucrase activity in fetal rat endoderm (Lacroix *et al.*, 1984b). Since during the course of the first trimester a progressive maturation of enzymatic function occurs in human fetal intestine, and a 22-week-old anencephalic fetus exhibited a significant reduction in brush border enzyme activities (Lacroix *et al.*, 1984 a), it was of some interest to determine whether this maturation occurring precisely between 8 and 14 weeks of gestation is hormone-dependent or not. It was found that the sole explantation of human fetal gut in a serum-free culture medium elicited a significant maturation (2-4-fold increase above preculture levels) of almost all enzyme activities (Ménard and Arsenault, 1985; Simon-Assmann *et al.*, 1986b). In order to be expressed, the overall maturation needs the presence of sugar (in particular glucose) in the culture medium. On the other hand, the addition of DX, insulin or amniotic fluid to the medium did not further enhance brush border enzyme activities except for lactase, whose levels were doubled by DX. Lactase activity is similarly stimulated by epidermal growth factor (Menard *et al.*, 1988).

Epithelial-mesenchymal interactions during development and crypt-villus epithelial maturation

In the digestive tract of birds and mammals the ontogenic regulation by tissue interactions of morphogenetic events and concomitant structural and functional differentiation has been increasingly studied since the pioneer work of Le Douarin *et al.* (1968). In several experimental models, grafted tissue recombinants were studied using either a structural cell marker system (Le Douarin, 1973) or tissue-specific marker molecules like brush-border hydrolases of the intestinal absorptive cells (Kedinger *et al.*, 1981) and pepsinogen of avian embryonic proventricular gland cells (Yasugi and Mizuno, 1981). They made it possible to define the role of mesenchyme and endoderm respectively in morphogenesis and epithelial cytodifferentiation. Permissive as well as instructive influences have been demonstrated during development of digestive anlagen (Fig. 4, for reviews see Kedinger *et al.*, 1986a, 1988a; Haffen *et al.*, 1987, 1989). Permissive influences are exemplified by homotopic mesenchymal effects that provide appropriate conditions for endodermal development and initial expression of species-specific intrinsic functional markers like sucrase and maltase (Fig. 4a, Kedinger *et al.*, 1981; Lacroix *et al.*, 1984b) or pepsinogen (Takiguchi *et al.*, 1988). Region-specific instructive influences exerted by digestive mesenchymes were demonstrated by their association with heterotopic endoderms followed by their graft into the coelomic cavity of chick embryos or explantation in organ culture. A peculiar example of this is provided by the intestinalization of gizzard endoderm in contact with intestinal mesenchyme (Fig. 4e). The occurrence of *de novo* synthesis of sucrase in conformity with enterocytic ultrastructural characteristics assessed either biochemically (Haffen *et al.*, 1982) or immunohistochemically (Ishizuya-Oka and Mizuno, 1984) strongly suggests that intestinal mesenchyme can control the differentiation process of stomach endoderm at the genomic level. In contrast, the morphogenetic effect of the proventricular mesenchyme assessed by the induction of gland formation in intestinal endoderm is not accompanied by the expression of pepsinogen, but rather shows sucrase immunoreactivity associated with ultrastructural enterocytic characteristics (Yasugi *et al.*, 1985). These results emphasize that in the digestive tract itself, the intestinal mesenchyme exerts the strongest inductive effect, whereas intestinal endoderm is rather resistant to inductive influences of heterologous mesenchymes, as first suggested by Gumpel-Pinot *et al.* (1978).

The persistence of epithelial-mesenchymal interactions during postnatal differentiation of the intestine has also been investigated in grafted cell and tissue associations. Two main sets of experiments have been designed to analyze the competence of adult epithelial crypt cells to respond to fetal mesenchymal influences as well as the instructive effect of intramucosal fibroblasts on the expression of epithelial differentiation. First, the IEC-17 cell line derived from rat intestinal crypt epithelium, established and characterized by Quaroni *et al.* (1979), is able to achieve a complete cytodifferentiation when associated with fetal intestinal mesenchyme (Fig. 4b, Kedinger *et al.*, 1986c). Second, an instructive role is played by postnatal rat intestinal fibroblasts which, like fetal intestinal mesenchyme, are able to change the fate of gastric

endoderm to an intestinal one (Fig. 4f, Haffen *et al.*, 1983). Such instructive properties are not carried by skin fibroblasts (Fig. 4g) which however exert a permissive action on endodermal tissue, no matter whether it is of intestinal (Fig. 4d, Kedinger *et al.*, 1988a) or stomach origin (Yasugi *et al.*, 1989). In turn, the intestinal endodermal cells induce the skin fibroblasts to differentiate into smooth muscle layers, as assessed by the expression of specific smooth muscle actin (Fig. 4h, Kedinger *et al.*, 1990).

The fact that heterotypic cell interactions are necessary for differentiating intestinal endodermal cells *in vitro* has also been stressed. Attempts to induce or maintain differentiated properties specific to absorptive cells in culture have all failed, except those where heterotypic cell contacts with mesenchymal elements have been maintained (for review see Kedinger *et al.*, 1987a). Indeed, the different behavior of embryonic intestinal endodermal cells cultured in isolation or overlayed on a confluent monolayer of fibroblasts, regardless of whether they are of intestinal or skin origin, demonstrates that epithelial cells survive, proliferate and differentiate only in coculture conditions (Kedinger *et al.*, 1987b; Stallmach *et al.*, 1989).

Evidence that mesenchyme influences and contributes to intestinal epithelial cell differentiation is further strengthened by the finding that glucocorticoid effects on the epithelial cells are mediated by the mesenchyme. The initial observation that intestinal epithelial cytodifferentiation is not only controlled by tissue interactions but also by hormones comes from the transplantation experiments of heterospecific epithelial-mesenchymal recombinants. Indeed, the more or less elevated levels of circulating hormones supplied by the recipient host (adult rat or chick embryo) lead to important variations in the degree of brush border enzyme maturation (Kedinger *et al.*, 1983, 1988b). The role of the mesenchyme in this hormonal effect is indicated by the following experiments. First, testing the morphological and functional maturation of recombinants composed of chick intestinal endoderm associated with different mesenchymal supports and their enzymatic response to glucocorticoids, Lacroix *et al.* (1985) have pointed out that a differential effect in glucocorticoid-evoked alkaline phosphatase activity is dependent upon the mesenchymal environment. The second observation concerns hybrid intestines composed of rat small intestinal mesenchyme and rat colonic endoderm. During the perinatal period in rodents there is a transient expression in the proximal colon of small intestinal-like brush border enzymes, which parallels the transient presence of villus structures; however, sucrase is not expressed and cannot be induced by glucocorticoids in organ culture. The combination of colonic endoderm with small intestinal mesenchyme does not influence the expression of the brush border enzyme pattern in the colonic endoderm; however glucocorticoids are able to induce the expression of sucrase in such recombinants explanted in organ culture (Foltzer-Jourdainne *et al.*, 1989). This observation suggests that the small intestinal mesenchyme, unlike the colonic mesenchyme, is able to mediate the effect of the hormones on sucrase induction. Finally, rat intestinal endodermal cells cultured in isolation do not respond to glucocorticoids while in endodermal cells cocultured on top of fibroblastic cells, the hormones enhance or induce expression of brush border hydrolases (Kedinger *et al.*, 1987b).

The mesenchymal mediation of glucocorticoid action on intestinal epithelial cells is further confirmed by the finding that glucocorticoid receptors are located at early fetal stages within the intestinal mesenchyme mostly in the cell layers in the immediate vicinity of the endoderm (Kedinger *et al.*, 1989).

Extracellular matrix molecules in the developing intestine

As in several other cell systems, the concept emerges that in dynamic epithelial-mesenchymal interactions, as well as in mesenchyme dependence on hormone stimulation, the transfer of permissive or inductive signals can occur via changes in the extracellular matrix (ECM) environment. ECM is a complex network of molecules forming the ground substance, the connective tissue, in which cells are embedded. Some of these constituents are assembled in a specialized structure, the basement membrane (BM) that underlies epithelial, endothelial and certain mesenchymal cells (Hay, 1981; Schuppan and Hahn, 1987; Timpl, 1989). In the intestine, laminin, nidogen, undulin, type IV collagen, heparan sulfate proteoglycan and hyaluronate are found almost homogeneously all along the crypt-villus axis at the epithelial-lamina propria interface (Fig. 2). In addition to their localization in the BM region, these molecules, except undulin, as well as other interstitial proteins, fibronectin, tenascin and collagens type I, III, V, VI surround cellular elements in the lamina propria (Quaroni *et al.*, 1978; Laurie *et al.*, 1982; Simon-Assmann *et al.*, 1986a, 1989; Hahn *et al.*, 1987a; Hayashi *et al.*, 1987; Aufderheide and Ekblom, 1988; Alho and Underhill, 1989; Schilt *et al.*, 1990).

The possible involvment of ECM molecules in cell interactions is suggested by the observation of changes in their deposition and distribution in the developing rat or mouse intestine (Simon-Assmann *et al.*, 1986a; Aufderheide and Ekblom, 1988) as well as during the spontaneous or triiodothyronine-induced metamorphosis in amphibian intestine (Schilt *et al.*, 1990). Moreover, quantitative and qualitative modifications of ECM, mainly BM molecules like type IV collagen (Simon-Assmann *et al.*, 1990) and laminin (Simo *et al.*, in preparation), are obvious at critical stages of intestinal morphogenesis. In addition, several experimental data emphasize that ECM components could provide the structural basis for intestinal differentiation. Elaboration of a BM comprising type IV collagen, laminin, nidogen and heparan sulfate proteoglycan is obvious when epithelial cells are cocultured with fibroblastic cells but never when one or the other cell population is cultured in isolation (Fig. 5, Hahn *et al.*, 1987b; Kedinger *et al.*, 1987c; Simon-Assmann *et al.*, 1988). The deposition of the BM molecules at the epithelial-mesenchymal interface occurs progressively and precedes expression of epithelial cell differentiation markers (Kedinger *et al.*, 1988b, 1989). Taking advantage of chick-rat epithelio-mesenchymal hybrid intestines in which BM components have been analyzed with species-specific antibodies, it could be clearly shown that the mesenchymal cells are the principal endogenous source of type IV collagen (Fig. 6a and b; Simon-Assmann *et al.*, 1988). This conclusion is further confirmed by the observation of specific type IV collagen mRNA accumulation within the fetal mesenchyme and later on within the lamina propria (Fig. 6c; Weiser *et al.*, 1989; Simon-Assmann *et al.*, 1990). The biochemical analysis of laminin synthesis also suggests that this molecule is mainly produced by the fibroblastic cell compartment (Simo *et al.*, in preparation), a result that is confirmed by the preferential localization of laminin transcripts in the intestinal mesenchyme-derived compartments (Senior *et al.*, 1988; Weiser *et al.*, 1989). In contrast, the production and deposition in the BM of heparan sulfate proteoglycan molecules are achieved by the

epithelial cell compartment, as shown with species-specific antibodies in interspecies epithelial-mesenchymal tissue recombinants (Simon-Assmann *et al.*, 1989). Furthermore, the expression of tenascin in the mesenchyme is dependent on the presence of epithelial cells, as shown by transfilter cultures (Aufderheide and Ekblom, 1988).

Finally, arguments in favor of glucocorticoid effects on ECM components arose from epithelial-fibroblastic coculture experiments in which the synthetic pattern of glycosaminoglycans has been analyzed with respect to epithelial differentiation (Bouziges *et al.*, 1989). These experiments lead to the conclusion that glucocorticoids act, at least partly, through modifications in the nature and distribution of the glycosaminoglycans synthesized by the fibroblastic cells. Similarly a redistribution of laminin molecules is induced by glucocorticoids in monocultures of fibroblastic cells, where the proportion of cell-associated molecules is increased to the detriment of those secreted into the medium; in parallel, laminin deposition at the epithelial-fibroblastic interface in cocultures is accelerated by the hormones (unpublished results). *In vivo*, glucocorticoid-induced precocious maturation of suckling rat intestine is associated with a differential regulation of the gene expression of interstitial and basement membrane collagens, fibronectin and laminin. In particular, administration of glucocorticoids increases the amounts of fibronectin, type IV procollagen, and laminin as well as of their respective mRNAs. Interestingly, hormone administration to adult rats, whose intestinal-specific enzymes are no longer affected by the hormone, results in insignificant changes in intestinal ECM mRNAs (Walsh *et al.*, 1987).

Taken together, these data emphasize a cellular cooperation for the elaboration of a precise ECM microenvironment, including the basement membrane at the stromal-epithelial interface, which in turn appears to be of prime importance in directing morphological changes and subsequent gene expression (Fig. 7a). In addition, the finding that most of the ECM components modified by glucocorticoids *in vivo* and *in vitro* are produced by the mesenchymal-derived compartment strengthens the idea that this latter cell population represents the actual target cells of glucocorticoids. These cells respond to the hormones by modifying their synthetic pattern of ECM molecules, thus providing a microenvironment allowing the process of epithelial differentiation to be achieved (Fig. 7b).

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